Remarks

Claims 1-6, 10, 11, 12, 21, 23, 26 and 28 having been amended and claims 7 and 37 having been cancelled herewith, the pending claims are claims 1-6, 10-15, and 17-36. Of these, claims 1, 2, 12, 13, 15, 22, and 24-35 have been withdrawn from examination, such that claims 3-6, 10, 11, 14, 17-21, 23, and 36 are presently under examination.

Claims 1, 3-5, 10 and 11 have been amended to recite an isolated nucleic acid molecule. Support for this amendment is found in the specification at, for example, page 20, line 20.

Claim 4 has been amended to recite a membrane targeting portion of an i-antigen polypeptide which is capable of targeting a polypeptide to the endoplasmic recticulum or to the plasma membrane. Support for this amendment is found in the specification at, for example, page 18, lines 12-14.

Claims 6 and 21 have been amended to recite a *Tetrahymena* host cell. Support for this amendment is found throughout the specification, for example at page 25, lines 23-28, and in the claims as originally filed, for example claim 7.

Claim 10 has been amended to recite exemplary hybridization conditions as found in the specification at page 17, lines 23-26.

Claim 11 has been amended to recite exemplary hybridization conditions as found in the specification at page 18, lines 15-16.

Claim 2 has been amended to correct an obvious error in the dependency of the claim.

Claims 12, 26 and 28 have been amended to correct obvious grammatical errors.

Claim 7, reciting several organisms including *Tetrahymena*, and claim 37, reciting a multicellular organism, have been canceled, without prejudice, as claim 6 from which they depend has been amended to recite a *Tetrahymena* host cell.

Reconsideration and withdrawal of the rejections in view of the above amendments and the following comments are respectfully requested.

Errors to the Appendix to the Amendment and Response filed June 3, 2002

Applicants respectfully draw the Examiners attention to claims 2, 6, 12, 13, and 22 recited in the Appendix to the Amendment and Response filed June 3, 2002.

Claim 2 was originally filed as depending from "claim 2," and was corrected to depend from claim 1 in the Appendix submitted June 3, 2002. However, the change was made without indicating an amendment had been made. Applicants have made this amendment in the present Amendment and Response.

Additionally, through oversight, Applicants inadvertently set forth incorrect text for claims 12, 13, and 22 (presently withdrawn from examination) in the Appendix to the Amendment and Response submitted June 3, 2002. Applicants have, in the present Amendment, set for the correct text for claims 12, 13, and 22, which is the text as originally filed. No amendments have been made to claims 13 and 22. As noted above, claim 12 has been amended herewith to correct an obvious grammatical error.

Status of Claims

In the Office Action mailed April 9, 2003, the Examiner noted in the section entitled, "Detailed Action" that claims 1-7, 10-15 and 17-37 were pending in the instant application, and further noted that claims 1-2, 12-13, 15, 22, and 24-35 had been withdrawn from further consideration as being drawn to a non-elected invention. Consequently, claims 3-7, 10-11, 14, 17-21, and 36-37 were under consideration.

Applicants note with appreciation that the Examiner has agreed to include claims 5 and 11 in the group of claims presently under examination, pursuant to Applicants' request set forth in the response submitted June 3, 2002.

Additionally, Applicants note that while the paragraph under the heading "Detailed Action" of the Office Action mailed April 9, 2003 indicates claim 23 as pending, it is neither indicated as withdrawn, allowed, or rejected in this paragraph. As Item 6 of the Office Action Summary indicates claim 23 is rejected, Applicants proceed on the understanding that the claim

23 is pending and currently under examination. Clarification is requested if this assumption is incorrect.

Rejection under 35 U.S.C. §101

The Examiner rejected claims 3-5, 10-11, 14, 17, 19, and 36 under 35 U.S.C. § 101, alleging the claimed invention is directed to non-statutory subject matter.

This rejection is respectfully traversed. However, in order to further prosecution of the instant patent application, Applicants have amended claims 3-5, 10 and 11 to recite an "isolated" nucleic acid molecule and respectfully submit that this amendment overcomes this rejection. Furthermore, the rejections to claims 14, 17, 19, and 36, dependent on amended claims, are also overcome by these amendments. Reconsideration and withdrawal of the rejection of claims 3-5, 10-11, 14, 17, 19, and 36 is, therefore, respectfully requested.

Rejection under 35 U.S.C. §112, First Paragraph

The Examiner rejected claims 6, 21, and 37 under 35 U.S.C. § 112, first paragraph, alleging the specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims. Specifically, the Examiner alleged that the specification is enabling for a *T. thermophila* host cell transformed with a heterologous DNA construct, but does not reasonably provide enablement for a transgenic cell. This rejection is respectfully traversed.

Applicants note at the outset that claim 37 is cancelled, thereby rendering the rejection moot with respect to this claim.

Applicants respectfully disagree with the Examiner's position that the specification does not provide support for a transgenic cell other than a *T. thermophila* cell. However, solely for the purpose of advancing prosecution of the above-identified patent application, claims 6 and 21 are amended to recite a "*Tetrahymena* host cell" (see, e.g., specification at page 25, lines 23-28 and original claim 7).

Applicants respectfully submit that claims 6 and 21, as amended, are enabled by the specification as originally field. Reconsideration and withdrawal of the rejection of claims 6, 21, and 37 is therefore respectfully requested.

The Examiner rejected claims 3, 4, 6, 7, 10, 11, 14, 17-21, 23, and 37 under 35 U.S.C. § 112, first paragraph, alleging these claims contain subject matter which as not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor had possession of the claimed invention at the time the application was filed. This written description rejection is respectfully traversed.

Applicants note at the outset that claims 7 and 37 are cancelled, thereby rendering the rejection moot with respect to those claims.

Of the rejected claims, claims 3, 4, and 11 are independent. Claim 3, as amended, recites:

> 3. An isolated nucleic acid molecule comprising a polynucleotide fragment having a nucleotide sequence that encodes an antigenic portion of an i-antigen polypeptide having amino acid sequence SEQ ID NO:7, said antigenic portion of the i-antigen polypeptide comprising at least about 60 amino acids and being capable of inducing an immune response in a fish against *I. multifiliis*.

Claim 4, as amended, recites:

> 4. An isolated nucleic acid molecule comprising a polynucleotide fragment having a nucleotide sequence that encodes at least one terminal membrane targeting portion of an i-antigen polypeptide having amino acid sequence SEQ ID NO:7, said terminal membrane targeting portion comprising at least about 10 amino acids and being capable of targeting a polypeptide to either the endoplasmic recticulum or to the plasma membrane.

Claim 11, as amended, recites:

> 11. An isolated nucleic acid molecule comprising a polynucleotide fragment that hybridizes to at least a portion of the complement of SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:44 or SEQ ID NO:102 under conditions comprising about 150 mM NaCl, 15 mM trisodium citrate, and pH 7.6 at 55°C, wherein the

polynucleotide fragment encodes a polypeptide comprising at least a membrane targeting portion or an antigenic portion of an i-antigen protein, wherein said antigenic portion is capable of inducing an immune response in a fish.

Claims 6, 10, 14, 17-21 and 23 depend directly or indirectly from, *inter alia*, claims 3 and 4.

The Examiner characterizes claims 3, 4, 6, 7, 10, 11, 17-21, and 37 as reciting a nucleic acid molecule encoding an antigenic protein or peptide fragment. This is only partially correct. Applicants wish to clarify that the nucleic acid molecule of claim 3 encodes "an antigenic portion of an i-antigen polypeptide having amino acid sequence SEQ ID NO:7" and that the nucleic acid molecule of claim 4 encodes "a terminal membrane targeting portion of an i-antigen polypeptide having amino acid sequence SEQ ID NO:7." The nucleic acid molecule of claim 11 encodes a membrane targeting portion or an antigenic portion of an i-antigen protein, and the nucleic acid molecule hybridizes to defined nucleotide sequences under defined conditions.

In further defining the structure of the claimed nucleic acid molecule, claims 3 and 4 additionally recite that the portion of the i-antigen polypeptide encoded by the nucleic acid molecule includes at least 10 (claim 4) or 60 (claim 3) amino acids of SEQ ID NO:7.

Claims 3 and 4 also recite functional characteristics of the claimed nucleic acid molecules. Specifically, the portion of SEQ ID NO:7 that is encoded by the nucleic acid molecule is "antigenic" (claim 3) or a "terminal membrane targeting" portion (claim 4). The antigenic portion is further characterized functionally in claim 3 as "capable of inducing an immune response in a fish against *I. multifiliis*"; likewise, the terminal membrane targeting portion is further characterized functionally in claim 4 as "capable of targeting a polypeptide to either the endoplasmic recticulum or to the plasma membrane". Claim 11 recites that the antigenic portion is capable of inducing an immune response in a fish.

The Examiner argues that the claimed genus is "highly variant" and that, as a result, SEQ ID NO:7 alone is insufficient to describe the genus. The Examiner further alleges that Applicants have not described a function which is "shared by SEQ ID NO:7" which would adequately describe the genus, and contends that the disclosure fails to provide a representative

number of species to describe the genus. Applicants respectfully disagree with the Examiner's analysis.

As noted above, claims 3, 4 and 11 recite, for each claimed nucleic acid, both structure and related function that characterize the genus. Additionally, one of skill in the art can readily determine whether a nucleic acid molecule that possesses the recited structure also possesses the recited function by reference to the specification and/or the general knowledge in this highly skilled art. At page 19, lines 16-23, for example, the specification provides an assay to determine antigenicity, stating:

> An antigenic analog, fragment, or modification of a polypeptide having SEQ ID NOs:6 or 7 is one that generates an immune response in fish against *I. multifiliis*. Antigenicity of an [sic] polypeptide can be evaluated *in vitro* by performing a Western blot on the purified polypeptide (for example, an affinity purified polypeptide) using polyclonal antisera from a rabbit that was vaccinated with at least an antigenic portion of a native *I. multifiliis* i-antigen protein, preferably with a complete *I. multifiliis* i-antigen protein (e.g., SEQ ID NO:6 or SEQ ID NO:7).

The ability of an antigenic polypeptide to generate an immune response in fish can be determined using the procedures set forth in Examples 6 and 7, for example, describing the generation of an immune response in channel catfish using a protein subunit (Example 6) or DNA (Example 7) vaccine. Membrane targeting sequences are readily identifiable, for example, using comparative sequence analysis as N-terminal and C-terminal (GPI-anchor) targeting sequences have well-known sequence homology. For example, the specification at page 44, lines 10-20, teaches one method for identifying an N-terminal membrane targeting sequence:

> . . . [N]eural network algorithms trained on signal peptides and their cleavage sites (H. Nielsen et al., Prot. Engin., 10:1-6 (1997)) were used to examine the first 50 amino acids of the deduced protein sequence beginning with the methionine residue cited above. Such algorithms identified the first 20 amino acids as a signal peptide (S mean = 0.839), and predicted a cleavage site between the alanine and valine residues (amino acids 20 and 21, respectively) of the deduced

> amino acid sequence. The N-terminal amino acid of the 48 kD antigen protein corresponds to the valine residue predicted above (Clark et al., Proc. Nat. Acad. Sci. USA, 89:6363-6367 (1992)). Taken together, these observations argue strongly that amino acids 1-20 of the deduced protein constitute a signal peptide.

C-terminal membrane targeting sequences are likewise well known in the art and readily identifiable from the literature, as described, for example, in the specification at page 41, lines 28-31, to page 42, lines 1-8:

> Hydrophobic domains at the C-termini of GPI-anchored proteins are necessary for covalent attachment of glycosylphosphatidylinositol moieties (P. Englund, Annu. Rev. Biochem., 62:121-138 (1993)), and the deletion of relevant coding sequences from transgenes that encode such proteins usually results in secretion rather than membrane binding. Furthermore, there are a number of instances in which alternative splicing of endogenous mRNAs gives rise to transcripts that either specify, or fail to encode hydrophobic C-terminal peptides (I. Caras et al., Nature, 325:545-548 (1987); H. Gower et al., Cell, 55:955-964(1988)). In the first case, such products are retained at the plasma membrane (as GPI-anchored proteins), while in the second, they are exported from the cell. Both secreted and membrane bound forms of a 48 kDa i-antigen have been described in *I. multifiliis* (C. Xu et al., J. Euk. Microbiol., 42:558-564 (1995)).

SEQ ID NO:7, the amino acid sequence of the full 55kD i-antigen, is antigenic (see, e.g., Example 6, page 56, lines 21-24 of the specification) and contains both an N-terminal (specification at page 14, lines 21-29) and C-terminal (specification at page 14, lines 29-31, to page 15, lines 1-11) membrane targeting sequence. The genus of nucleic acid molecules to which claims 3 and 4 are directed encodes a polypeptide which includes one or the other functionally characterized portions of SEQ ID NO:7. The recited functionality of the polypeptide portion is readily assayable and, in each case, is a function shared by the full length polypeptide represented by SEQ ID NO:7. Likewise, the nucleic acid molecule of claim 11 is defined with reference to a particular structure (SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:44 or SEQ ID NO:102) and a function that is known to be shared by i-antigen polypeptides.

It is therefore respectfully submitted that the claims subject to the rejection recite a partial structure together with related functional characteristics that are clearly defined and assayable. Applicants contend that they are in possession of the necessary common attributes or features of the elements possessed by the members of the genus in view of the species disclosed and claimed, in accordance with the Written Description Guidelines promulgated in the Federal Register, Vol. 64, No. 244, pages 71427-71440 on December 21, 1999. It is respectfully submitted that independent claims 3, 4, and 11, as well as claims 6, 10, 14, 17-21, and 23, dependent therefrom, comply with the written description requirement of 35 U.S.C. § 112, first paragraph.

In view of amendments to the claims and the comments presented herein, reconsideration and withdrawal of the rejection of claims 3-4, 6-7, 10-11, 14, 17-21, 23, and 37 under 35 U.S.C. §112, first paragraph are respectfully requested.

Rejection under 35 U.S.C. §112, Second Paragraph

The Examiner rejected claims 3 and 4 under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. This rejection is respectfully traversed.

The Examiner stated that the claims are vague and indefinite in the recitation of "at least about," asserting that the term confers two separate contradictory limitations. Applicants respectfully disagree.

Claim 3 recites a molecule including a polynucleotide fragment having a nucleotide sequence encoding an antigenic portion of an i-antigen polypeptide including at least about 60 amino acids. The encoded portion may include more than 60 amino acids; therefore, in essence, about 60 amino acids is a lower end of a range. The term "about" indicates there is some tolerance at the lower end of the range. Claim 4 recites similar language, reciting that the terminal portion of the i-antigen polypeptide includes at least about 10 amino acids. Applicants assert, therefore, that no contradictory limitations are indicated by the use of the term "at least about" in claims 3 and 4.

Furthermore, recitation of the term "at least about" does not render the claim *per se* indefinite. *Chemical Separation Technology Inc. v. United States*, 63 U.S.P.Q.2d 1114, 1123 (US CtFedCls 2002), *citing*, *Amgen, Inc. v. Chugai Pharmaceutical Co., Ltd.*, 927 F.2d 1200, 18 U.S.P.Q.2d 1016 (Fed. Cir. 1991) (the ruling that the term "at least about 160,000" was indefinite . . . "should not be understood as ruling out any and all uses of this term in patent claims"). (*See also*, *In re Wertheim, et al.*, 191 U.S.P.Q. 90, 96 (C.C.P.A. 1976) "It is not necessary that the application describe the claim limitations exactly . . . but only so clearly that persons of ordinary skill in the art will recognize from the disclosure that appellants invented processes including those limitations."). Applicants respectfully assert that one skilled in the art would understand that the recitation of the term "at least about" in claims 3 and 4 indicates a tolerance in what is in essence the lower end of a range.

The Examiner rejected claim 10 under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. Specifically, the Examiner alleged that the term "substantially complementary" is not clearly defined in the claims, although the Examiner admitted in the present Office Action at page 7, last paragraph, that "substantially complementary" is defined in detail at page 17, line 24 to page 18, line 6 of the specification. Applicants respectfully submit that the definition of "substantially complementary" may be construed in light of the specification ("it is fundamental that claims are to be construed in the light of the specifications and both are to be read with a view to ascertaining the invention" *United States v. Adams et. al.*, 148 U.S.P.Q. 479, 482 (1966)). However, solely for the purpose of advancing prosecution of this application, Applicants have amended claim 10 to recite an isolated nucleic acid molecule that hybridizes with any of the nucleic acid molecules of claims 3-5 or 36 under conditions exemplified by about 150 mM NaCl, 15 mM trisodium citrate, and pH 7.6 at 55°C. Applicants respectfully submit that the amendment to claim 10 overcomes this rejection.

The Examiner rejected claim 11 under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. Specifically, the Examiner alleged that the conditions of "standard hybridization" are not clearly defined. Applicants respectfully disagree, particularly as conditions for standard hybridization are clearly set forth in the specification at, for example, page 18, lines 15-16. However, solely to advance prosecution of the present application, Applicants have amended claim 11 to recite hybridization conditions, as disclosed in the specification at page 18, lines 15-16, including about 150 mM NaCl, 15 mM trisodium citrate, and pH 7.6, at 55°C. Applicants respectfully submit that this amendment overcomes the Examiner's rejection of claim 11.

In view of amendments to the claims and the arguments presented above, withdrawal of the rejection of claims 3, 4, 10, and 11 under 35 U.S.C. §112, second paragraph, is respectfully requested.

Rejection under 35 U.S.C. §102(b)

The Examiner rejected claims 4, 6-7, 10-11, 14, 17, 19, and 21 under 35 U.S.C. §102(b) as being anticipated by Clark et al. (PNAS, 89:6363-6367 (1992)). This rejection is respectfully traversed.

The Examiner is requested to note at the outset that the cancellation of claim 7 renders the rejection moot as to this claim.

"A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference." MPEP § 2131.

Clark et al. teach the polynucleotide sequence of a cDNA encoding a portion of a 48 kDa i-antigen from an *I. multifiliis* G1 isolate, serotype A. The Clark et al. nucleotide sequence (Exhibit A) represents a portion of nonelected SEQ ID NO:6 (specifically, amino acids 22 through 409 of SEQ ID NO:6 as shown in Fig. 1 of the present application, a copy of which, with the nucleotides indicated, is included herewith and marked as Exhibit B, compared with

amino acids 1-389 of Clark et al.). Clark et al. do not teach a 55 kDa antigen derived from an *I. multifiliis* G5 isolate or a polynucleotide sequence encoding SEQ ID NO: 7. In particular, Clark et al. do not teach a polynucleotide sequence encoding at least one terminal membrane targeting portion of an antigen polypeptide having SEQ ID NO:7 which includes at least about 10 amino acids, as recited in claim 4, or an antigen portion of an i-antigen polypeptide having SEQ ID NO:7 which includes at least 60 amino acids.

It is clear from Fig. 3(a) (a copy of which is included herewith and marked as Exhibit C) of the present application, for example, that the 55 kDa i-antigen from a G5 isolate of *Ichthyophthirius* (SEQ ID NO: 7), which is the basis of the elected invention, is a different polypeptide from the 48 kDa i-antigen of Clark et al. The 48 kDa i-antigen and the 55 kDa i-antigen are each encoded by different polynucleotide sequences. See also page 29, lines 25-30 of the specification.

Turning now to the Sequence Search conducted by the Examiner and attached to the Office Action mailed April 9, 2003, the following comments are made. This Sequence Search was conducted to compare SEQ ID NO:7 (the 55kD i-antigen) with a database sequence (the 48 kD i-antigen sequence of Clark et al.). However, the comparison begins with amino acid 93 of SEQ ID NO:7. Thus, the N-terminal sequence of SEQ ID NO:7 was not even used in making this sequence comparison. For the record, it is also noted that the database sequence used as the reference sequence actually begins with amino acid 22 of the full 48 kD i-antigen sequence (SEQ ID NO:6). In comparing the sequence of Clark et al (the database sequence) with a portion of SEQ ID NO:7, there is only one common group of at least about 10 consecutive amino acids (circled on the copy of the Sequence Search included herewith and marked as Exhibit D), which are nucleotides 165-176 of SEQ ID NO:7. Applicants respectfully submit that nucleotides 165-176 are not located toward an extremity (i.e., a terminal portion) of a sequence of 468 nucleotides (SEQ ID NO:7).

Furthermore, Clark et al., do not teach a polynucleotide fragment that hybridizes to at least a portion of the complement of SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 44, or SEQ ID NO: 102 (i.e., SEQ ID NO: 7), under standard hybridization conditions, wherein the

polynucleotide fragment encodes a polypeptide including at least a membrane targeting portion or an antigenic portion of an i-antigen protein, wherein said antigenic portion is capable of inducing an immune response in fish, as recited in claim 11 and the claims dependent therefrom.

Clark et al. therefore does not disclose each and every element as set forth in the Applicants' claims. Reconsideration and withdrawal of the rejection under 35 U.S.C. §102(b) is, therefore, respectfully requested.

Summary

It is respectfully submitted that pending claims 3-6, 10, 11, 14, 17-21, 23, and 36, currently under examination, are in condition for allowance, and notice to that effect is earnestly solicited. The Examiner is invited to contact Applicants' Representative at the below listed number if it is believed that prosecution of the above-identified application can be in any way assisted or expedited thereby.

Respectfully submitted,

Clark et al.

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CERTIFICATE UNDER 37 CFR §1.10::
"Express Mail" mailing label number: EV 073 687 979 US Date of Deposit: October 8, 2003
I hereby certify that the Transmittal Letter and the paper(s) and/or fee(s), as described hereinabove, are being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR §1.10 on the date indicated above and is addressed to the Assistant Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

By: _____
Name: Rachel Gagliardi-Grabau

ffinity chromatography with a mouse monoclonal antibody (designated 10H3) that strongly immobilized the *Ich* isolate used in these studies (18). After purification by SDS/PAGE, the N-terminal amino acid sequence of the 48-kDa antigen was determined by Edman degradation using Applied Biosystems 470A/477A automated protein sequencers (see Fig. 1). An "antisense" 24-mer oligonucleotide probe (5'-AGCAGCACCAACATCAGTCAAACC-3') corresponding to eight amino acids (Gly-Leu-Thr-Asp-Val-Gly-Ala-Ala) near the N terminus of the protein was synthesized, end-labeled with ^{32}P, and used to screen the cDNA library (19). Among a number of positive recombinants isolated, one (designated clone 2-3) contained a 1.2-kilobase (kb) *Eco*RI insert and was further analyzed. The full-length *Eco*RI insert and individual *Pst* I restriction fragments were subcloned in M13 phage, and the 1.2-kb cDNA was sequenced in its entirety off both strands by the dideoxy method of Sanger *et al.* (20).

Northern Hybridization Analysis. Parasites were concentrated by centrifugation for 2 min at 1000 × *g* and total RNA was isolated from cell pellets after lysis in guanidine thiocyanate (21). Poly(A)$^+$ RNA was purified by two rounds of chromatography on oligo(dT)-cellulose. Final concentrations of total and poly(A)$^+$ RNA were determined by absorbance at 260 nm. RNA was fractionated by electrophoresis on 1.2% agarose gels containing 2.2 M formaldehyde and transferred to Biotrace nylon filters (Gelman) in 20× SSC (3 M NaCl/0.3 M sodium citrate, pH 7.0). Filters were hybridized with alkali-denatured probe (≥10^6 cpm/ml), then washed at 65°C as described by Mahmoudi and Lin (22). Final wash buffer was 40 mM Na_2HPO_4/1% SDS/1 mM EDTA, pH 7.2. For preparation of the probe, the 1.2-kb cDNA insert was purified by agarose gel electrophoresis and labeled to >10^9 cpm/μg with [α-^{32}P]dATP by using random oligonucleotide priming (23).

Quantitation of RNA Transcripts. In all cases, RNA levels were measured under hybridization conditions of probe excess. Changes in transcript prevalence during development were determined from densitometry scans of autoradiographic exposures of Northern blots in which equal amounts of RNA from different stages of the parasite were loaded. X-ray film strips with exposures in the linear range of response of the film were scanned spectrophotometrically at 470 nm and individual peak areas were measured and compared. Results were normalized to ethidium fluorescence in ribosomal RNA bands or to signals generated by hybridization of the same filters with a heterologous actin cDNA under conditions of reduced stringency (24).

Absolute levels of RNA were determined by using a single-stranded RNA probe as described in Fig. 3. For probe synthesis, the 1.2-kb cDNA was subcloned in pBluescript by *in vivo* excision from λ ZAP II DNA according to the supplier (Stratagene), and the orientation of the cDNA insert was determined by restriction endonuclease analysis. Antisense RNA was then synthesized under standard conditions from the T7 promoter flanking the insert (25) and the template was removed by digestion with RQ1 DNase (Promega).

Southern Hybridization Analysis. Total genomic DNA was isolated from *Ich* tomites by methods previously described for *Tetrahymena* (26). This DNA was considered to be predominantly macronuclear in origin by analogy with other holotrich ciliates (27). DNA was digested to completion and

SEQ ID NO:6 MS 22-409

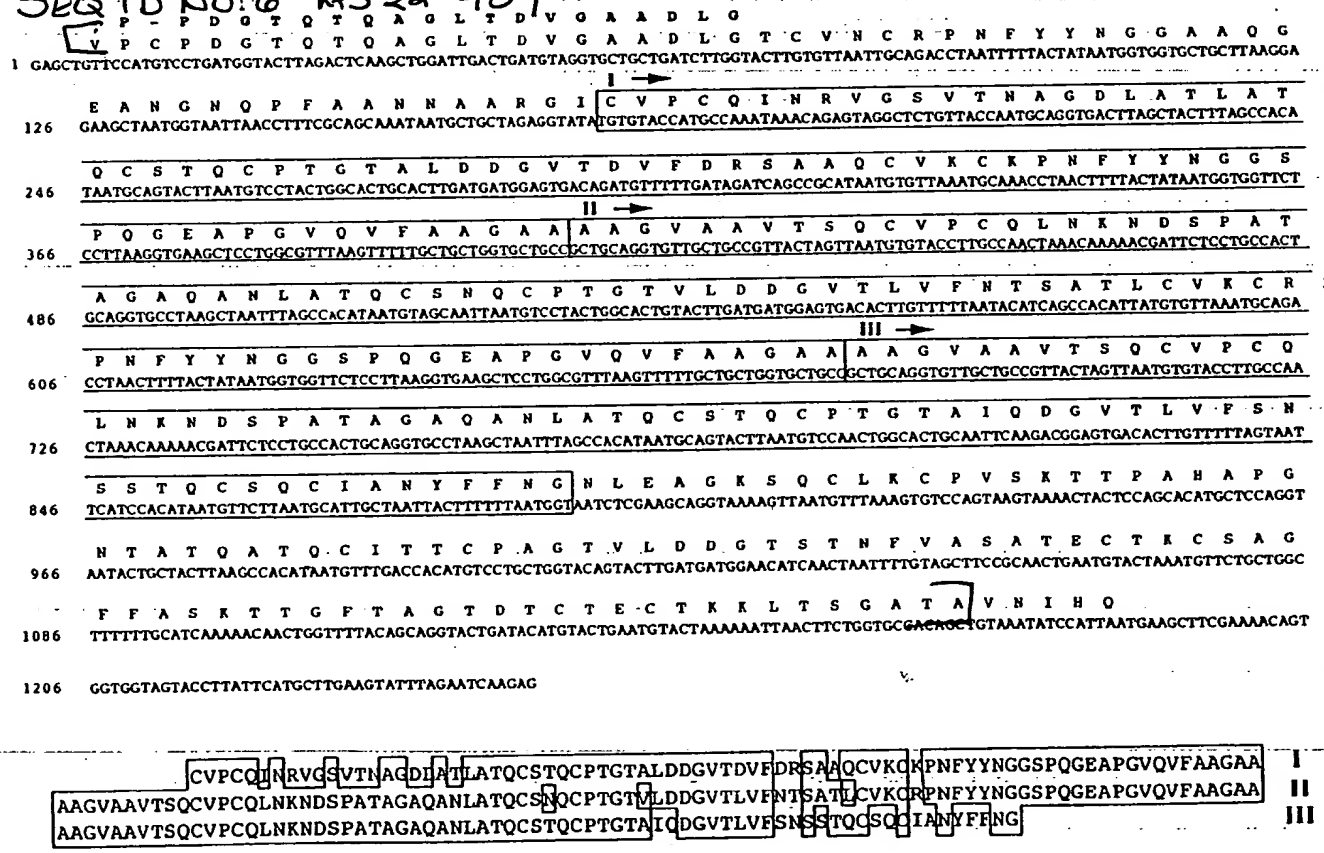

FIG. 1. Nucleotide and deduced amino acid sequence of the *Ich* i-antigen cDNA. The complete nucleotide and deduced amino acid sequence of the 1.2-kb i-antigen cDNA is shown at the top. The N-terminal amino acid sequence of the 48-kDa antigen is on the first line; hyphens represent amino acids that could not be identified with certainty. The areas in the sequence that are boxed and designated by Roman numerals show the regions of extended homology within the deduced protein structure, and they are aligned relative to one another at the bottom part of the figure. Domains I and II, and II and III share 82% and 83% identity at the amino acid sequence level, respectively.

Exhibit A

48 kD G1 i-antigen: protein and nucleic acid sequences

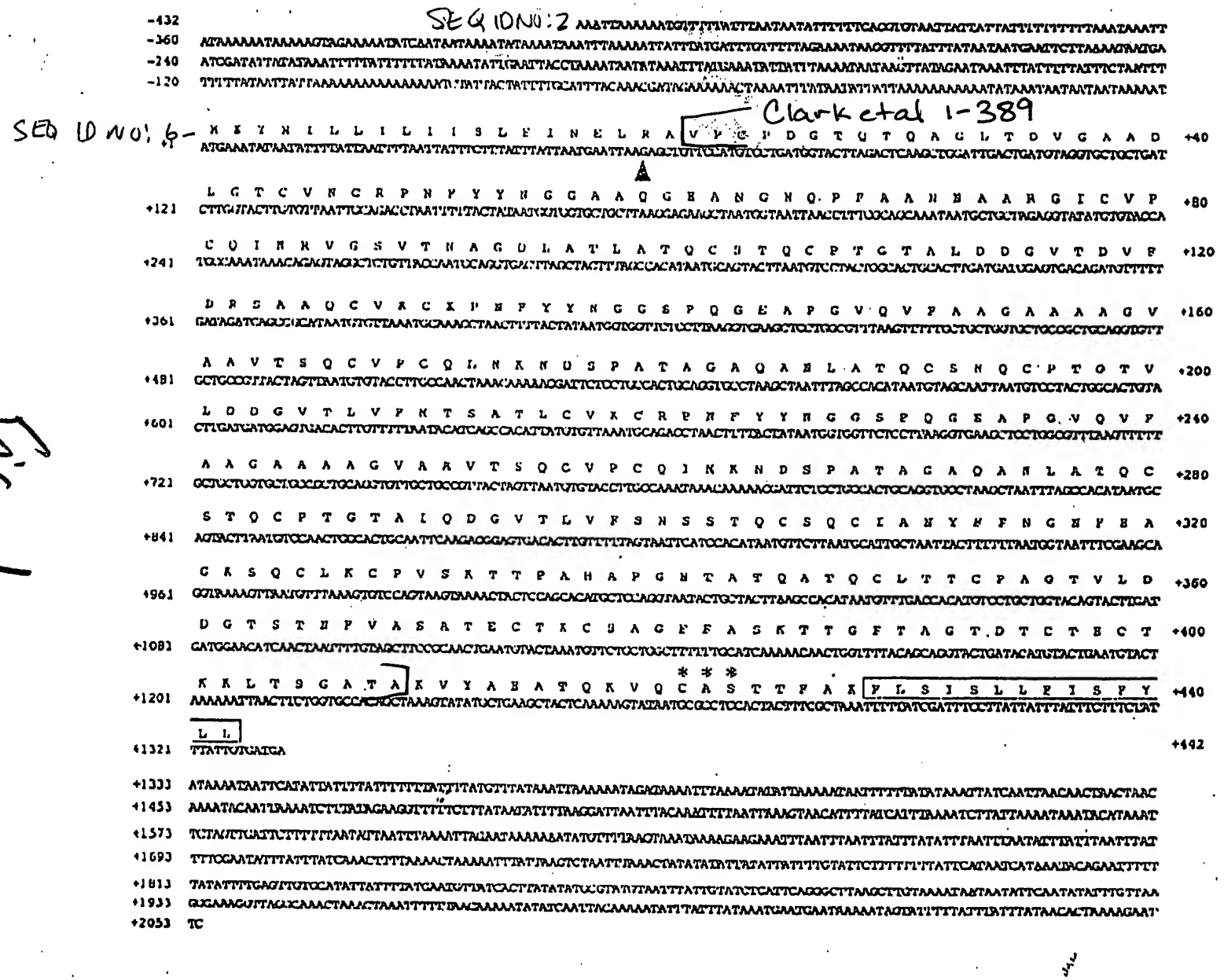

Fig. 1

Exhibit B

Sequence alignment of 48 kD G1 i-antigen and 55 kD G5 i-antigen protein sequences

Nucleotide 2 of Clark et al;
1 Starting point of that SEQUENCE IN THE SEQUENCE SEARCH

SEQ ID NO: 6 G1
SEQ ID NO: 7 G5

```
G1   1 MKYNILLILIISLFINELRAVPCPDGTQT-QAG-LTDVGAADLGTCVNCRPNFYYN----
G5   1 MKNNILVILIISLFINQIKSANCPVGTETNTAGQVDDLGTP--ANCVNCQKNFYYNNAAA
       ** ***:********** :::: . ** **:*  ** : *:*:.  ..****: *****

G1  55 --GGAA------QGEANGNQP-------------------------------------------
G5  59 FVPGASTCTPCPQKKDAGAQPNPPATANLVTQCNVKCPAGTAIAGGATDYAAIITECVNC
          **:      * :  * **

G1  68 ---F----AAN-NAARGICVPCQINRVGSVTNAGDLATLATQCSTQCPTGTALDDGVTDV
G5 119 RINFYNENAPNFNAGASTCTACPVNRVGGALTAGNAATIVAQCNVACPTGTALDDGVTTD
          *    *.* **. . *..* :****.. .**: **:.:**..  ***********

G1 120 FDRSAAQCVKCKPNFYYNGGSPQGEAPGVQVFAAGAAAAGVAAVTSQCVPCQLNK--NDS
G5 179 YVRSFTECVKCRLNFYYNGNN--GNTP----FNPGK---------SQCTPCPAIKPANVA
       : ** :: ****: ******..  *::*    * .*          ***.**   *  * :

G1 178 PATAGAQANLATQCSNQCPTGTVLDDGVTLVFNTSATLCVKCRPNFYYNGGSPQGEAPGV
G5 224 QATLGNDATITAQCNVACPDGTISAAGVN-NWVAQNTECTNCAPNFYNNN------AP--
        ** * :*.:::**.  ** **:   **.  : :. * *.:* **** *.      **

G1 238 QVFAAGAAAAGVAAVTSQCVPCQINKND-SPATAGAQANLATQCSTQCPTGTAIQDGVTL
G5 295 -NFNPG---------NSTCLPCPANKDYGAEATAGGAATLAKQCNIACPDGTAIASGATN
         * .*         .* *:**  **:  : ****. *.**.**.  ** **** .*.*

G1 297 VFSNSSTQCSQCIANYFFNG-NFEAGKSQCLKCPVSKTTPAHAP-GNTATQATQCLTTCP
G5 325 -YVILQTECLNCAANFYFDGNNFQAGSSRCKACPANKVQGAVATAGGTATLIAQCALECP
       :   .*:* :* **::*:* **:**.*:*  **..*.  * *. *.***  :**   **

G1 355 AGTVLDDGTSTNFVASATECTKCSAGFFASKTTGFTAGTDTCTECTKKLTSGATAKVYAE
G5 384 AGTVLTDGTTSTYKQAASECVKCAANFYTTKQTDWVAGIDTCTSCNKKLTSGAEANLPES
       ***** ***:.:.  :*:**.**:*.*:::* *.:.** **** * ******* *:  .

G1 415 ATQKVQCASTTFAKFLSISLLFISFYLL
G5 444 AKKNIQCD---FANFLSISLLLISYYLL
       *.:::**     **.*******.**.***
```

SEQ ID NO: Conserved regions SEQUENCE SEARCH

```
90 MKYNILLILIISLFIN
91 MKNNILVILIISLFIN
```

↑93 - Starting point of Query (Q4) of the SEQUENCE Search

```
92 CPTGTALDDGVT
```

```
93 CVKCKPNFYYNG
94 CVKCRLNFYYNG
```

```
95 CPAGTVLDDGT
96 CPAGTVLTDGT
```

```
97 AGTDTCTECTKKLTSGATA
98 AGIDTCTSCNKKLTSGAEA
```

```
99  FAKFLSISLLFISFYLL
100 FANFLSISLLLISYYLL
```

Fig. 3(a)

Exhibit C

```
Db    268 ------------------PGPNSKCVACESKKT-NSQSRSGLEANLAAQCGTECPAGTL 307

Qy    318 IASGAT-NYVILQTECLNCAANFYFDGNNFQAGSSRCKACPANKVQGAVATAGGTATLIA 376
          :  | |  | :  ::|:|| | ||   :||:|| |:|  |  :|  |: :  | :||
Db    308 VTDGVTPTYTVSLSQCVNCKAGFY-QNSNFEAGKSQCNKCAVSKT-GSASVPGNSATSAT 365

Qy    377 QCALECPAGTVLTDGTTSTYKQAASECVKCAANFYTTKQTDWVAGIDTCTSCNKKLTSGA 436
          ||  :|||||||: |||:: :   |||| || |||| :| : : || |||| |:|||||||
Db    366 QCQNDCPAGTVVDDGTSTNFVALASECTKCQANFYASKTSGFAAGTDTCTECSKKLTSGA 425

Qy    437 EANLPESAKKNIQC---DFANFLSISLLLISYYLL 468
            | :   | :  ||    || |||:||: ||:|||
Db    426 TAKVYAEATQKAQCASSTFAKFLSMSLIFISFYLL 460
```

RESULT 4
Q27208
ID Q27208 PRELIMINARY; PRT; 395 AA.
AC Q27208;
DT 01-NOV-1996 (TrEMBLrel. 01, Created)
DT 01-DEC-2001 (TrEMBLrel. 19, Last sequence update)
DT 01-JUN-2002 (TrEMBLrel. 21, Last annotation update)
DE Immobilization antigen precursor (Fragment).
OS Ichthyophthirius multifiliis.
OC Eukaryota; Alveolata; Ciliophora; Oligohymenophorea; Hymenostomatida;
OC Ophryoglenina; Ichthyophthirius.
OX NCBI_TaxID=5932;
RN [1]
RP SEQUENCE FROM N.A.
RC STRAIN=GEORGIA;
RX MEDLINE=92335298; PubMed=1631132;
RA Clark T.G., McGraw R.A., Dickerson H.W.;
RT "Developmental expression of surface antigen genes in the parasitic
RT ciliate Ichthyophthirius multifiliis.";
RL Proc. Natl. Acad. Sci. U.S.A. 89:6363-6367(1992).
RN [2]
RP SEQUENCE FROM N.A.
RC STRAIN=GEORGIA;
RX MEDLINE=93020590; PubMed=1383510;
RA Lin T.L., Dickerson H.W.;
RT "Purification and partial characterization of immobilization antigens
RT from Ichthyophthirius multifiliis.";
RL J. Protozool. 39:457-463(1992).
RN [3]
RP SEQUENCE FROM N.A.
RC STRAIN=GEORGIA;
RA Clark T.;
RL Submitted (SEP-1998) to the EMBL/GenBank/DDBJ databases.
DR EMBL; M92907; AAC36158.1; -.
DR PRINTS; PR01574; TUBBYPROTEIN.
KW Signal.
FT NON_TER 1 1
FT SIGNAL <1 1 POTENTIAL.
FT CHAIN 2 395 IMMOBILIZATION ANTIGEN.
SQ SEQUENCE 395 AA; 39567 MW; 68DA2C790E4FD393 CRC64;

Query Match 30.5%; Score 775.5; DB 5; Length 395;
Best Local Similarity 44.2%; Pred. No. 7.9e-49;
Matches 175; Conservative 35; Mismatches 129; Indels 57; Gaps 16;

SEQ ID NO:7 Clark et al.

```
Qy     93 VKCPAGTAIAGGATDY-AAIITECVNCRINFY----------NENAP--NFNAGASTCTA 139
          | || ||    | ||  || : ||||| |||           | | |    ||    |
Db      2 VPCPDGTQTQAGLTDVGAADLGTCVNCRPNFYYNGGAAQGEANGNQPFAANNAARGICVP 61

Qy    140 CPVNRVGGALTAGNAATIVAQCNVACPTGTALDDGVTTDYVRSFTECVKCRLNFYYNGNN 199
          | :||||    ||: ||:  ||:  ||||||||||||  : ||  :||||: |||||| :
Db     62 CQINRVGSVTNAGDLATLATQCSTQCPTGTALDDGVTDVFDRSAAQCVKCKPNFYYNGGS 121

Qy    200 --GNTP----FNPG---------KSQCTPCPAIKPANVAQATLGNDATITAQCNVACPDG 244
            |  |    | |           ||| ||   |  | : || |  | :  ||:  || |
Db    122 PQGEAPGVQVFAAGAAAAGVAAVTSQCVPCQLNK--NDSPATAGAQANLATQCSNQCPTG 179

Qy    245 TISAAGVNNWVAQNTE---CTNCAPNFYNN------NAPN---FNPG---------NSTC 283
```

```
          |:   ||   :  ||    |  | |||| |       ||    |  |          | |
Db    180 TVLDDGVT--LVFNTSATLCVKCRPNFYYNGGSPQGEAPGVQVFAAGAAAAGVAAVTSQC 237

Qy    284 LPCPANKDYGAEATAGGAATLAKQCNIACPDGTAIASGAT-NYVILQTECLNCAANFYFD 342
          :||  ||:  : ||||  | || ||:  || ||||  | |  :    |:|  | ||::|:
Db    238 VPCQINKN-DSPATAGAQANLATQCSTQCPTGTAIQDGVTLVFSNSSTQCSQCIANYFFN 296

Qy    343 GNNFQAGSSRCKACPANKVQGAVATAGGTATLIAQCALECPAGTVLTDGTTSTYKQAASE 402
          | | :|| |:|  || :|   | |  | |||    ||   |||||| |||:: :  :|:|
Db    297 G-NLEAGKSQCLKCPVSKTTPAHA-PGNTATQATQCLTTCPAGTVLDDGTSTNFVASATE 354

Qy    403 CVKCAANFYTTKQTDWVAGIDTCTSCNKKLTSGAEA 438
          | ||:| |: :| | : || |||| | |||||| |
Db    355 CTKCSAGFFASKTTGFTAGTDTCTECTKKLTSGATA 390
```

RESULT 5
Q9GPP0
ID Q9GPP0 PRELIMINARY; PRT; 371 AA.
AC Q9GPP0;
DT 01-MAR-2001 (TrEMBLrel. 16, Created)
DT 01-MAR-2001 (TrEMBLrel. 16, Last sequence update)
DT 01-MAR-2001 (TrEMBLrel. 16, Last annotation update)
DE Immobilization antigen LD (Fragment).
GN SERLD.
OS Tetrahymena thermophila.
OC Eukaryota; Alveolata; Ciliophora; Oligohymenophorea; Hymenostomatida;
OC Tetrahymenina; Tetrahymena.
OX NCBI_TaxID=5911;
RN [1]
RP SEQUENCE FROM N.A.
RC STRAIN=ANF18211;
RX MEDLINE=20549003; PubMed=11095959;
RA Doerder F.P., Gerber C.A.;
RT "Molecular Characterization of the SerL Paralogs of Tetrahymena
RT thermophila.";
RL Biochem. Biophys. Res. Commun. 278:621-626(2000).
DR EMBL; AF312775; AAG38107.1; -.
FT NON_TER 1 1
SQ SEQUENCE 371 AA; 35175 MW; 5817EFFC2517DEAC CRC64;

Query Match 13.6%; Score 345; DB 5; Length 371;
Best Local Similarity 30.1%; Pred. No. 1.6e-17;
Matches 141; Conservative 41; Mismatches 175; Indels 112; Gaps 33;

```
Qy      8 ILIISLFINQIKSAN-CPVGTETNTAGQVDDLGTPANCVNCQKNFYYNNAAAFVPGASTC 66
          :::||| :  | : | |   |:||       |    |  |   :|  :      ||  |
Db      6 LILISLAV--IATVNAC---TDTNATA-----GAGGTCF-CNAGYYGTSTDVTASGA--C 52

Qy     67 TPCPQKKDAGAQPNPPATANLVTQCNVKCPAGTAIAGGATDYAAIITECVNCRINFYNEN 126
            ||   :: |     |:  ||| |          ||   | :        |: |||
Db     53 QKCPTGTNSVA---ATASGTLVTSCT----CNDTNAGLKADNSG-----CQCKANFY--G 98

Qy    127 APNFNAGAST-CTACPVNRVGGALTAGNAATIVAQCNVACPTGTALDDGVTTDYVRSFTE 185
            ||  || :| |||||     | |  || ||      ||              |   ::
Db     99 TPNAVAGGATGCTACP---TGTASPAGTAAVTSCACN-----------DTNASLKGDNS 143

Qy    186 CVKCRLNFYYNGNNGNTPFNPGKSQCTPCPAIKPANVAQATLGNDATITAQCNVACPDGT 245
            :|: |||   |       | : || ||       : |  |: |  :  ||      |
Db    144 GCQCKANFYGTPN----AVAGGATGCTACP-----TGSAAAAGSTAVTSCACN-----DT 189

Qy    246 ISAAGVNNWVAQNTECTNCAPNFYNNNAPNFNPGNST-CLPCPANKDYGAEATAGGAATL 304
            ||        | |: |  |  |||     ||    | :| |  ||      |: | ||  |
Db    190 NSAL-----KADNSACI-CKANFY--GTPNAVAGGATGCTACPT----GSAAAAGSTAVT 237

Qy    305 AKQCNIACPDGTAIASGATNYVILQTECLNCAANFYFDGNNFQAGSSRCKACPANKVQGA 364
          :  ||       |  |  | |     : |: | ||||   |    |:: | |||     |
Db    238 SCACN-----DTNSALKADN-----SACI-CKANFYGTPNAVAGGATGCTACPT----GT 282

Qy    365 VATAGGTATLIAQCALECPAGTVLTDGTTSTYKQAASECVKCAANFYTTKQTDWVAGIDT 424
          :|||   |:|  ||  ||        | ::   |     :| |||| |  |   :|
Db    283 TSTAG--TTVIGSCA--CP-------DTNASLNTATPPVCQCNANFYGTPTTTGASG--- 328
```